VIROLOGY 229, 360–369 (1997)
ARTICLE NO. VY978443

Binding of Antibodies to Virion-Associated gp120 Molecules of Primary-like Human Immunodeficiency Virus Type 1 (HIV-1) Isolates: Effect on HIV-1 Infection of Macrophages and Peripheral Blood Mononuclear Cells

LEONIDAS STAMATATOS,*,[1] SUSAN ZOLLA-PAZNER,†‡ MIROSLAW K. GORNY,‡ and CECILIA CHENG-MAYER*

*Aaron Diamond AIDS Research Center, The Rockefeller University, New York, New York 10021-6399; †Veterans Administration Medical Center, New York, New York 10010; and ‡New York University Medical Center, New York, New York 10016

Received October 3, 1996; returned to author for revision December 5, 1996; accepted January 7, 1997

Using immunobiochemical approaches we previously studied the conformation and surface exposure of different immunodominant regions within the oligomeric, virion-associated form of the gp120 envelope glycoprotein of the human immunodeficiency virus type 1 (HIV-1) (L. Stamatatos and C. Cheng-Mayer (1995) *J. Virol.* 69, 6191–6198). These studies allowed us to determine to what extent epitopes within these immunodominant regions of the oligomeric gp120 are occluded or accessible to antibody binding on the virion surface of two primary-like (HIV-1_{SF162} and HIV-1_{SF128A}) and one T-cell-line-adapted (HIV-1_{SF2}) isolates. Here, we investigate whether binding of anti-gp120 monoclonal antibodies (MAbs) to exposed epitopes of the immunodominant regions of oligomeric gp120 results in neutralization of HIV-1 infection and whether certain exposed sites are better targets for neutralization than others. We also test whether proposed mechanisms for antibody-mediated neutralization of T-cell-line-adapted HIV-1 isolates, e.g., antibody-mediated gp120-virion dissociation, are applicable to primary-like HIV-1 isolates. We assess the neutralization potential of anti-V2 loop, anti-V3 loop, and anti-CD4 binding site MAbs using human primary macrophages or peripheral blood mononuclear cells (PBMC) as target cells and HIV-1_{SF162} and HIV-1_{SF128A} as infecting isolates. Our data indicate that: (i) not every exposed epitope of the immunodominant regions of gp120 oligomers on the virion surface is a target for neutralization; (ii) during virus–cell fusion specific exposure of previously occluded V3 loop epitopes within gp120 oligomers occurs, which may become targets for neutralization; (iii) antibody-mediated gp120-virion dissociation does not appear to be a major mechanism of neutralization for the primary-like HIV-1 isolates tested here; and (iv) infection of macrophages is more sensitive to neutralization by anti-gp120 monoclonal antibodies than PBMC. We also report that binding of one of the two anti-CD4 binding site MAbs tested mediated enhancement of macrophage cell infection in a concentration-dependent fashion, while binding of the other anti-CD4 binding site MAb resulted in efficient neutralization of infection of both macrophages and PBMC.

INTRODUCTION

In an effort to understand how the structure of the envelope glycoprotein of the human immunodeficiency virus type 1 (HIV-1) regulates certain biological properties of this virus, such as its cellular tropism, cytopathology, and neutralization sensitivity to antibodies and recombinant soluble receptor (sCD4), we began probing the conformation of the gp120 envelope subunit of isolates displaying distinct phenotypes *in vitro* using immunochemical approaches (Harrowe, 1995; Koito *et al.,* 1995; Stamatatos and Cheng-Mayer, 1993, 1995; Stamatatos *et al.,* 1994). We determined the relative binding affinity (based on half maximal antibody–virion binding values) and maximal extent of binding (antibody bound to virions at saturation) of monoclonal antibodies (MAbs) to the V2 loop (MAbs G3.4 and G3.136), the V3 loop (MAbs 257D, 268D, and 391-95D), and the CD4-binding site (MAbs 654-30D and 559-64D) of virion-associated gp120 molecules (Stamatatos and Cheng-Mayer, 1995). These studies revealed that in the absence of virion-CD4 binding, virion-associated gp120 molecules of primary-like macrophage-tropic HIV-1 isolates are oligomerized in a way that certain V3 loop epitopes are occluded and inaccessible to MAb binding, in accordance with reports from other investigators (Bou-Habib *et al.,* 1994; Sullivan *et al.,* 1995). This observation contrasts with that made using laboratory-adapted, T-cell-line-tropic viruses whose V3 loop appears exposed and accessible to MAb binding on virions and infected T-cell-lines (Bou-Habib *et al.,* 1994; Sattentau and Moore, 1995; Stamatatos and Cheng-Mayer, 1995; Sullivan *et al.,* 1995). Contrary to the occluded nature of their V3 loop, the V2 loop and CD4-binding site of macrophage-tropic primary-like isolates are accessible to antibody binding (Stamatatos and Cheng-Mayer, 1995).

Here we attempted to correlate the virion-binding properties of the MAbs mentioned above with their neutralization potential, in both PBMC and macrophages, against two primary-like macrophage-tropic HIV-1 isolates, HIV-1_{SF162} and HIV-1_{SF128A}. Our goal was to begin to under-

[1] To whom correspondence and reprint requests should be addressed at Aaron Diamond AIDS Research Center, 455 First Ave., 7th floor, New York, NY 10016. Fax: (212) 725-1126. E-mail: leonidas@adarc.org.

0042-6822/97 $25.00

stand the mechanisms whereby primary HIV-1 isolates, which may be relatively resistant to antibody and sCD4 neutralization (Cheng-Mayer *et al.,* 1988; Moore *et al.,* 1992, 1995; Moore and Ho, 1995; Sawyer *et al.,* 1994; Sullivan *et al.,* 1995) can in fact be neutralized by certain MAbs.

Several studies have previously examined the mechanisms of antibody- and sCD4-mediated neutralization for T-cell-line-adapted HIV-1 isolates. These studies have suggested correlations between the neutralization potential of MAbs (or sCD4) with: (i) their association rate constant (Sattentau and Moore, 1995), (ii) their dissociation rate constant (VanCott *et al.,* 1994), (iii) their ability to promote gp120-virion-dissociation (Poignard *et al.,* 1995), and (iv) the degree of exposure of the epitopes they recognize on the oligomeric form of the envelope (Bou-Habib *et al.,* 1994; Sattentau and Moore, 1995; Sullivan *et al.,* 1995). In this study we tested to what degree the proposed neutralization mechanisms for T-cell-line-adapted isolates might also apply to primary-like HIV-1 isolates, because we believe that elucidation of the mechanisms by which antibodies neutralize primary HIV-1 isolates is central to the development of effective anti-HIV-1 vaccine strategies.

MATERIALS AND METHODS

Viruses and antibodies

Molecular clones of HIV-1_{SF162} and HIV-1_{SF128A} isolates were used. The biological properties of these isolates have been previously reported (Cheng-Mayer *et al.,* 1990; Liu *et al.,* 1990). Briefly, HIV-1_{SF162} and HIV-1_{SF128A} do not infect established T-cell-lines and are relatively resistant to serum and sCD4 neutralization (Cheng-Mayer *et al.,* 1988; Stamatatos *et al.,* 1994). The viruses were generated by transfection of the viral DNA into RD-4 cells or 293-T cells and cocultivation with PHA-stimulated (3 μg/ml for 3 days) human peripheral blood mononuclear cells (PBMC) as previously reported (Cheng-Mayer *et al.,* 1990). Human primary macrophages were prepared by the plastic adherence method as previously described (Cheng-Mayer *et al.,* 1989) with the only modification being that the wells were coated with polylysine (Sigma, St. Louis, MS) prior to addition of monocyte cells. The isolation and characterization of the human anti-V3 MAbs, 391-95D, 257D, and 268D, human anti-CD4 binding site MAbs, 654/30D and IgG1b12, were previously described (Burton *et al.,* 1994; Gorny *et al.,* 1993; Laal *et al.,* 1994). The murine anti-V2 MAbs, G3.4 and G3.136, were characterized previously (Fung *et al.,* 1992) and were generously provided by Taxon Biosystems Inc. (Houston, TX). The human anti-CD4 binding site MAb IgG1b12 was provided by Dennis Burton (The Scripps Research Institute, La Jolla, CA). The human anti-gp41 MAb 2F5 was provided by Alexandra Trkola (ADARC, New York, NY), and the 50-69 MAb was obtained from the NIH AIDS research and Reference Reagent Program. The anti-gp120 and anti-gp41 sheep polyclonal antibodies, D6205 and D6204, respectively, were purchased from International Enzymes, Inc. (Fallbrook, CA).

Quantitation of monoclonal antibody binding to intact virions, gp120 monomers, and antibody-mediated dissociation of gp120 molecules from the virion surface

Quantitation of MAbs bound to intact virions and soluble gp120 molecules was performed by an assay we described previously (Stamatatos and Cheng-Mayer, 1995). Briefly, aliquots of sucrose purified virions are divided into two equal fractions. One fraction is first treated with detergent (1% NP-40) to generate soluble gp120 molecules and then incubated with MAbs (0.01 to 20 μg/ml) for 3 hr at 37°. Following that period, the gp120/MAb mixtures are added to 96-well plates, whose wells are precoated with sheep polyclonal antibodies (D6205) against the carboxyl terminal of gp120. MAbs associated with D6205 captured gp120 molecules are detected with the use of the appropriate alkaline phosphatase-coupled anti-MAb antibody (Zymed Laboratories Inc., South San Francisco, CA). Thereafter, with the use of an enzyme-linked immunosorbent assay (ELISA), the relative amounts of MAbs bound to gp120 are quantitated by determining the optical density at 490 nm ([OD_{490}]) with the appropriate secondary antibodies coupled to alkaline phosphatase (Stamatatos and Cheng-Mayer, 1995; Stamatatos *et al.,* 1994). The other fraction (intact virions) is directly incubated with MAbs as above. The relative quantities of virion-associated MAbs are determined following pelleting of the virions by centrifugation (15,000 *g* for 90 min at 4°). During this step the non-virion-associated MAbs (vast majority of the total MAb added to the virions) remains in solution, while the virion-bound MAbs are present in the virion pellet. The pelleted virions are then lysed with 1% NP-40 and the generated gp120-MAb soluble complexes are added to D6205-coated 96-well plates as described above.

Upon MAb-virion binding, some gp120–MAb complexes may dissociate from the virion surface. These soluble complexes are present in the supernatant of the pelleted virions and are separately captured on 96-well plates coated with D6205 as described above. Therefore the relative quantities of gp120 molecules that become dissociated from the virion surface following their association with MAbs can be determined with the use of the above ELISA assay (Stamatatos and Cheng-Mayer, 1995). For each MAb concentration added to the virions we determined both the amount of MAb present in the viral pellet and that found complexed with gp120 in the supernatant. Spontaneous release of gp120 molecules from the virion surface was determined by incubating the virions in the absence of MAb under the above described conditions. The amounts of gp120 present in the super-

natant of the viral pellet were determined as above and the recorded $OD_{490\,nm}$ values were substracted from those obtained when virions were incubated with MAb.

Quantitation of exposure of gp41 epitopes on the virion surface mediated by binding of anti-gp120 MAbs to intact virions

Sucrose-purified virions are incubated with increasing concentrations (0, 0.01, 0.1, 0.5, 1, and 10 μg/ml) of human anti-CD4-binding site MAbs (654-30D or IgG1b12) and a fixed concentration (1 μg/ml) of human anti-gp41 MAbs (50-69 or 2F5). The binding of anti-gp41 MAbs to the virion surface, as a function of the concentration of the anti-gp120 MAb concentration added, is determined as described above by capturing gp41–MAb complexes (following virion disruption by 1% NP-40) on wells coated with polyclonal anti-gp41 antibodies (D6204).

Neutralization of infection

(a) Macrophages. Viruses (100 $TCID_{50}$ units in 100 μl of cell medium) are preincubated with an equal volume of serially diluted MAbs (0.01 to 20 μg/ml) for 1 hr at 37°. The virus–MAb mixture is then incubated with human primary macrophage cells for 3 hr at 37°. The viral inoculum is subsequently removed and the cells are washed several times with Hank's buffer. Infection is determined by quantitating the p24 antigen concentration in the cell medium 7 (for HIV-1_{SF162}) and 10 (for HIV-1_{SF128A}) days later. The percentage neutralization of infection for each MAb concentration tested is determined by comparing the p24 concentration of the experimental control (i.e., macrophages infected by HIV-1 in the absence of MAbs) as follows:

$$\text{Percentage neutralization} = [(\text{control}) - (\text{experimental})/(\text{control})] \times 100,$$

where control is p24 concentration in wells where virus is incubated with macrophages in the absence of MAb and experimental is p24 concentration in wells where virus is incubated with macrophages in the presence of MAb.

A curve is then generated by plotting the percentage neutralization versus antibody concentration, and the antibody concentrations that reduce the infectivity by 90 and 50% as compared to control (IC90 and IC50, respectively) are presented in Table 2.

MAb-mediated enhancement of infection is indicated when (experimental) $>$ (control). We define MAb-mediated enhancement of HIV-1 (for a given MAb concentration) as at least a twofold increase in infection in the presence of MAb, as compared to control infections.

(b) Peripheral blood mononuclear cells. One hundred $TCID_{50}$ units in 50 μl were incubated with serial dilutions of an equal volume of MAb for 1 hr at 37°. The mixture was then added to PBMC (4×10^5 in 100 μl of cell medium). Triplicate wells of 96-well plates were used for each MAb concentration. In negative control wells, virus was incubated with cells in the absence of MAb. Seven days later, the p24 antigen concentration of each well was determined. The IC_{90} and IC_{50} values are determined as above.

RESULTS

Neutralization of PBMC and macrophage-infection by monoclonal antibodies to the third hypervariable region of gp120

We previously reported on the binding kinetics of three human anti-V3 loop MAbs (257D, 268D, and 391-95D) to virion-associated and soluble gp120 molecules from HIV-1_{SF162} and HIV-1_{SF128A} isolates (Stamatatos and Cheng-Mayer, 1995). These data are summarized in Table 1. Briefly, MAb 391-95D binds more extensively than MAb 257D on the surface of HIV-1_{SF162} and HIV-1_{SF128A} virions, while MAb 268D shows very poor binding reactivity against these two isolates. A preferential exposure of the epitope recognized by MAb 391-95D over those recognized by MAbs 257D and 268D is observed upon virion-sCD4 binding. Presently, we tested the neutralization potential of these MAbs against HIV-1_{SF162} and HIV-1_{SF128A} in PBMC and primary macrophages as cell targets. As summarized in Table 2, MAb 391-95D is more potent in neutralizing the infection of primary macrophage cells and PBMC by HIV-1_{SF162} and HIV-1_{SF128A} than MAb 257D. MAb 268D had no effect on viral infectivity. The neutralization potentials of MAbs 391-95D and 257D (based on the IC_{90} and IC_{50} values) against both isolates are stronger when the target cells are primary macrophages than PBMC (Table 2).

Neutralization of PBMC and macrophage-infection by monoclonal antibodies to the second hypervariable region of gp120

We previously reported on the binding of the murine anti-V2 loop MAbs G3.4 and G3.136 to soluble and virion-associated gp120 molecules of HIV-1_{SF162} and HIV-1_{SF128A} isolates (Stamatatos and Cheng-Mayer, 1995) and additional confirmatory data are shown in Fig. 1 and Table 1. Binding of MAb G3.136 to either isolate does not mediate gp120-virion dissociation (data not shown). In contrast, binding of MAb G3.4 to HIV-1_{SF162}, but not HIV-1_{SF128A}, mediates substantial release of gp120 molecules from the virion surface in the form of gp120-G3.4 complexes (Fig. 1). At 10 μg/ml MAb G3.4, between 40 and 50% of the virion-associated gp120 molecules that become associated with MAb G3.4 are released from the virion surface of HIV-1_{SF162} as gp120/MAb G3.4 complexes during a 3-hr incubation period. However, irrespective of their binding characteristics (maximal extent of binding, relative binding affinities, and gp120-virion dissociation

TABLE 1

Binding of MAbs to Intact HIV-1_{SF162} and HIV-1_{SF128A} Virions

MAb	gp120 region	Maximal binding[a]	sCD4 effect[b] (a)	sCD4 effect[b] (b)	Half-maximal binding[c]	
391-95D	V3 loop	0.3 (0.1)	0.05	>1.0	0.1 (0.025)	0.15 (0.025)
257D	V3 loop	0.15 (0.05)	ND	0.25	0.15 (0.05)	0.15 (0.05)
268D	V3 loop	ND	ND	ND	ND	ND
G3.4	V2 loop	1.5 (0.35)	0.1	0.1	1 (0.2)	1.5 (0.2)
G3.136	V2 loop	0.5 (0.18)	NT	NT	1.7 (0.3)	4.9 (0.25)
654-30D	CD4 binding site	0.9 (0.2)	NT	NT	0.3 (0.1)	0.4 (0.15)
IgG1b12	CD4 binding site	0.4 (0.1)	NT	NT	0.1 (0.025)	0.3 (0.1)

Note. NT, not tested; ND, not determined with accuracy because of poor MAb-virion reactivity.

[a] The maximal values (OD_{490nm}) of antibody binding to intact HIV-1_{SF162} virions shown here are the average and standard deviation (in parenthesis) from at least three independent experiments and were obtained at saturation of binding (10 μg/ml of MAb added to virions).

[b] Column (a) indicates the extent of MAb-virion binding (OD_{490nm}) when 0.1 μg/ml MAb were added to HIV-1_{SF162} virions in the absence of sCD4 and column (b) in the presence of 100 n*M* sCD4. Similar results were obtained with HIV-1_{SF128A} (Stamatatos and Cheng-Mayer, 1995).

[c] The half-maximal MAb-virion binding values (μg/ml) were obtained as described elsewhere (Stamatatos and Cheng-Mayer, 1995). The left column was obtained with HIV-1_{SF162} and the right column was obtained with HIV-1_{SF128A}. The values represent the average and standard deviation (in parenthesis) from at least three independent experiments.

capabilities), both G3.4 and G3.136 MAbs failed to neutralize infection of PBMC and primary macrophages by these two isolates to any significant extent (IC_{90} was not attained even at 20 μg/ml concentration) (Table 2).

TABLE 2

IC_{90} and IC_{50} Neutralization Values for the MAbs Tested[a]

	IC_{90}		IC_{50}	
	PBMC	Mϕ	PBMC	Mϕ
	HIV-1_{SF162}			
Anti-V3 MAbs				
391-95D	20	5	5	2.25
257D	—[b]	—	10	15
268D	—	—	—	—
Anti-V2 MAbs				
G3.4	—	—	—	—
G3.136	—	—	—	—
Anti-CD4 domain MAb				
IgGb12	1	0.1	0.1	0.01
	HIV-1_{SF128A}			
Anti-V3 MAbs				
391-95D	20	9	15	5
257D	—	—	10	10
268D	—	—	—	—
Anti-V2 MAbs				
G3.4	—	—	—	—
G3.136	—	—	—	—
Anti-CD4 domain MAb				
IgGb12	1	0.1	0.5	0.01

[a] The data indicate the concentration (μg/ml) of MAb required to reduce infection of peripheral blood mononuclear cells (PBMC) or macrophages (Mϕ) by 90% (IC_{90}) and 50% (IC_{50}). The values are the average of at least four independent experiments.

[b] —, IC_{qo} or IC_{50} not attained at 20 μg/ml.

Neutralization and enhancement of infection mediated by monoclonal antibodies to the CD4-binding site of gp120

The human MAbs IgG1b12 and 654-30D bind to the CD4-binding domain of gp120 and efficiently neutralize several laboratory adapted isolates (Burton *et al.,* 1994; Laal *et al.,* 1994; L. Stamatatos *et al.,* unpublished observations). In addition, MAb IgG1b12 was shown to be potent in neutralizing many primary isolates (Burton *et al.,* 1994). We previously reported on the binding of MAb 654-30D to HIV-1_{SF162} and HIV-1_{SF128A} (Stamatatos and Cheng-Mayer, 1995). Here we show that although the maximal extent of binding of MAb 654-30D to the virion surface is higher than that of MAb IgG1b12 (Fig. 2 and Table 1), the latter MAb binds to the surface of HIV-1_{SF162} virions with a three time higher relative affinity (based on half maximal MAb-virion binding values) than the former MAb (Table 1 and Fig. 2). The relative binding affinities of both MAbs toward HIV-1_{SF128A} are indistinguishable (Table 1). Neither MAb mediates significant gp120-virion dissociation, even at 10 μg/ml (Fig. 2). In neutralization assays, MAb IgG1b12 efficiently neutralized both HIV-1_{SF162} and HIV-1_{SF128A} isolates (Table 2). However, similar to the anti-V3 neutralizing MAbs (391-95D and 257D), MAb IgG1b12 is more effective in neutralizing infection of macrophages than PBMC. In contrast MAb 654/30D failed to inhibit the infection of PBMC and primary macrophages by these two macrophage-tropic viral isolates even when present at high concentrations (20 μg/ml). In fact, when primary macrophages were used as target cells, MAb 654-30D mediated enhancement of infection by both HIV-1_{SF162} and HIV-1_{SF128A} in a concentration-dependent fashion (Fig. 3). The extent of enhancement was less pronounced when PBMC were used as target cells

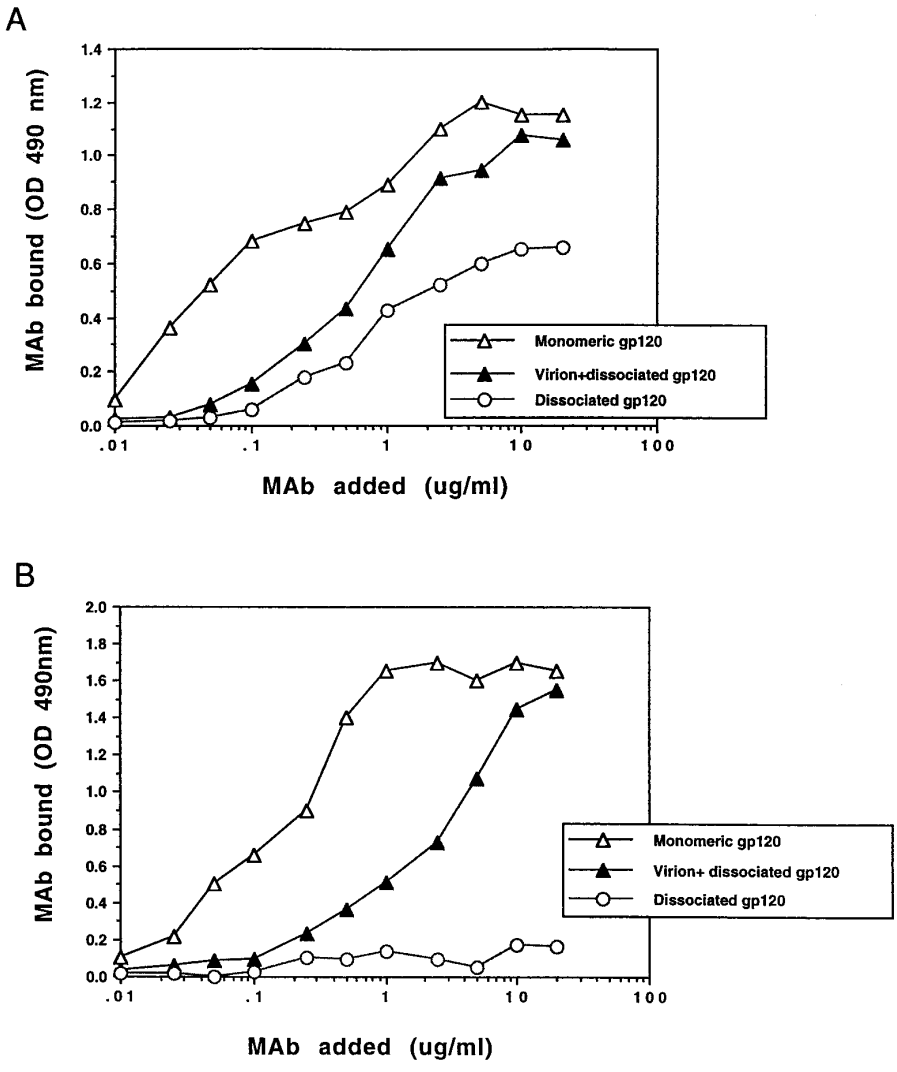

FIG. 1. Binding of anti-V2 loop MAb G3.4 to soluble and virion-associated gp120 molecules of (A) HIV-1_{SF162} and (B) HIV-1_{SF128A} isolates. Equal amounts of soluble and virion-associated gp120 molecules were incubated with increasing concentrations (0.1 to 20 μg/ml) of the murine anti-V2 loop MAb G3.4. The relative amounts of MAb G3.4 that became bound (OD 490 nm) to soluble gp120 or the virion surface, as well as the relative amounts of gp120 molecules that became dissociated from the virion surface following their interaction with MAb G3.4, were determined for each concentration of MAb G3.4 added. The results shown are representative of at least four independent experiments. The $OD_{490\,nm}$ values shown are corrected for nonspecific MAb binding to the wells (background).

(the maximal enhancement observed was less than two-fold as compared to the control infection, data not shown).

Gp41 epitope-exposure upon binding of anti-CD4 binding site MAbs to the surface of intact virions

To examine whether MAb 654-30D mediates enhancement of macrophage infection by inducing exposure of gp41 and thus facilitating virus–cell fusion, the binding of two anti-gp41 MAbs (50-69 and 2F5) to the virion surface before and during MAb 654-30D-virion association was determined (Fig. 4). MAb 2F5 neutralizes HIV-1 infection by primary HIV-1 isolates (Muster *et al.,* 1993). As a negative control we tested whether virion-binding of MAb IgG1b12 (which neutralizes infection of the isolates tested here (Table 2) mediates similar degree of exposure of the gp41 epitopes (Fig. 4). Our data show that neither MAb 654-30D nor MAb IgG1b12 mediated a significant increase in the binding of the two anti-gp41 epitopes examined (Fig. 4).

DISCUSSION

The studies described above were designed and carried out to determine whether the previously proposed mechanisms of antibody-mediated neutralization of T-cell line-adapted (TCLA) HIV-1 isolates apply to primary-like isolates. We observe that there is no simple predictive correlation between the virion-binding properties of MAbs and their neutralization potential against primary HIV-1 isolates. Furthermore, the exposure, or lack of ex-

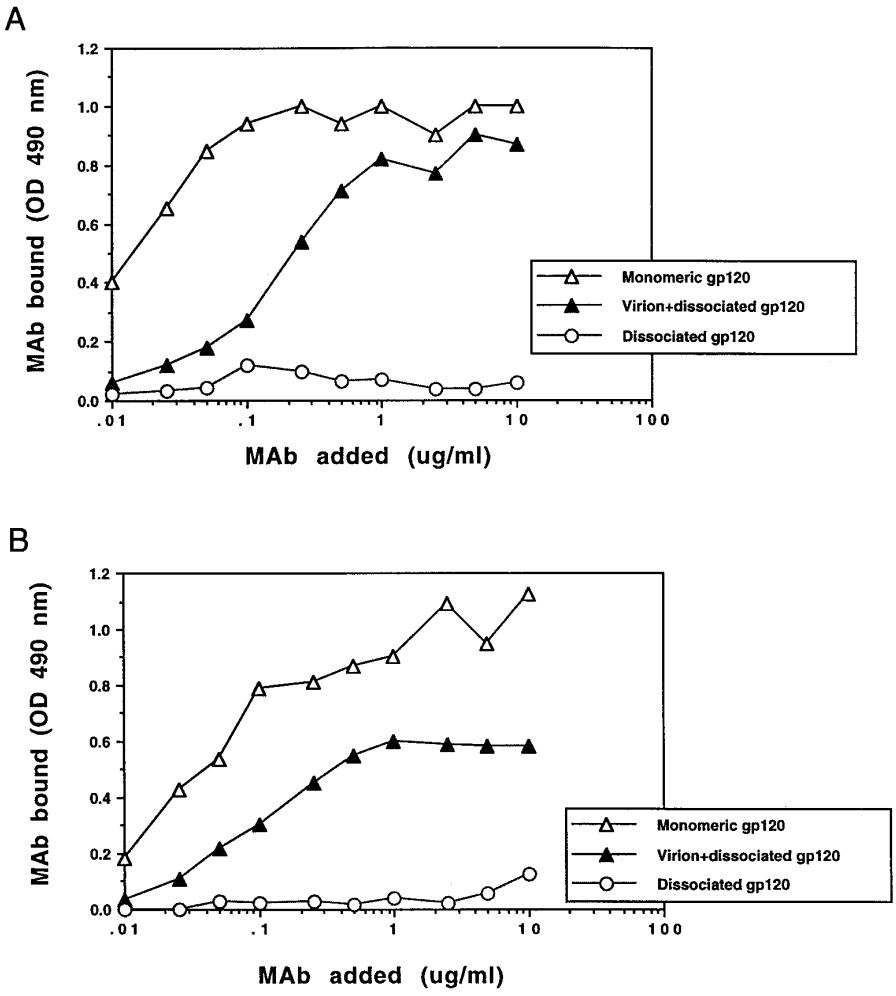

FIG. 2. Binding of anti-CD4-binding site MAbs to soluble and virion-associated gp120-molecules of HIV-1_{SF162} and MAb-mediated dissociation of gp120 molecules from virions. Equal amounts of soluble and virion-associated gp120 molecules were incubated with increasing concentrations of two human anti-CD4 binding site MAbs ((A) 654-30D and (B) IgG1b12) as described under Materials and Methods section. The amounts of MAb bound (OD 490 nm) to soluble gp120, to the virion surface, and those that were released from the virion surface in the form of MAb-gp120 complexes were determined for each MAb concentration added. The results shown are representative of at least five independent experiments. The $OD_{490\ nm}$ values shown are corrected for nonspecific MAb binding to the wells (background).

posure, of a gp120 epitope on the oligomeric gp120 envelope will not predict whether it will serve as a target for neutralization by antibodies. For example, the V3 loop epitopes recognized by MAbs 391-95D and 257D are known to be occluded within primary-like virion-associated envelope molecules (Table 1; Stamatatos and Cheng-Mayer, 1995), yet MAbs 391-95D and 257D do neutralize PBMC and macrophage infection by HIV-1_{SF162} and HIV-1_{SF128A} (Table 2). With respect to the anti-CD4-binding site MAbs tested, MAb IgG1b12 efficiently neutralized infection by HIV-1_{SF162} and HIV-1_{SF128A}, while MAb 654-30D failed to do so even though the former MAb bound less extensively to the virion surface than the latter MAb at saturation (Fig. 2). In addition, both anti-V2 MAbs failed to neutralize HIV-1 infection (Table 2) even though they both bound more extensively to the virion surface than the anti-V3 MAbs tested (Table 1; Stamatatos and Cheng-Mayer, 1995). The poor binding affinities of the anti-V2 loop MAbs to the oligomeric envelope of HIV-1_{SF162} and HIV-1_{SF128A} (Fig. 1, Table 1) might explain their poor neutralization potential (Sattentau and Moore, 1995). Other possibilities that cannot be excluded are that these two MAbs recognize epitopes that are, contrary to what was reported for TCLA viruses (Moore *et al.,* 1993), not involved during virus–cell fusion of primary-like HIV-1 isolates (see below), or less likely, that the epitopes recognized by G3.4 and G3.136 are only exposed on the surface of defective, noninfectious particles. Implicit to this last possibility is the requirement that the binding of some MAbs to noninfectious virion particles differs from that to infectious particles as a result of different gp120 conformations between these two populations of virions which are present in any viral preparation. At the present time it is not possible to determine the percentage of noninfectious virions which arise as a result of a defective envelope structure. However, previ-

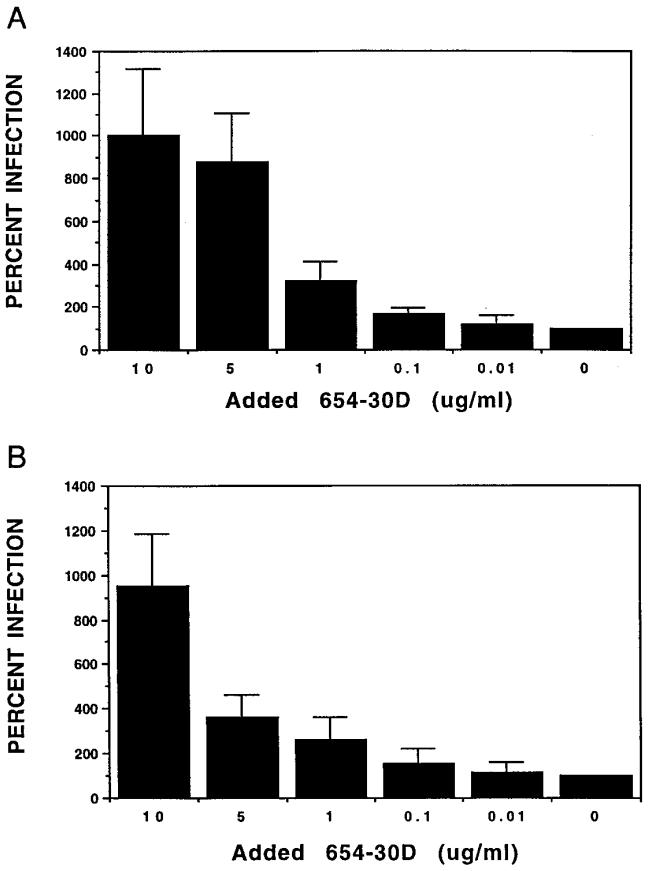

FIG. 3. Enhancement of macrophage cell infection by (A) HIV-1_{SF162} and (B) HIV-1_{SF128A} mediated by MAb 654-30D. Virus (100 $TCID_{50}$) was preincubated with serial dilutions of the anti-CD4 binding site MAb 654-30D and then added to primary human macrophage cells for 3 hr at 37° as described under Materials and Methods. The inoculum was removed and the cells were further cultivated for 7 (in the case of HIV-1_{SF162}) and 10 (in the case of HIV-1_{SF128A}) days at which point the p24 antigen concentration in the supernatant was determined. The p24 concentration was compared to control samples (100% infection), i.e., virus directly inoculated into macrophages without pretreatment with MAb 654-30D. The results shown represent the mean and standard deviation of at least four independent experiments.

ous studies (Bernier *et al.,* 1995), indicated that the majority of defective HIV-1 particles produced by two stable cell lines contained fully functional envelope proteins, but lacked reverse transcriptase and/or integrase proteins. In addition, the fact that MAbs IgG1b12 and IgG-CD4 (which recognize complex epitopes on gp120) bind to the same virion preparations used in the anti-V2 loop MAb binding studies and efficiently neutralize the infection of the same viruses (Table 2 and L. Stamatatos unpublished data), suggests that the major fraction of the viral population (both infectious and noninfectious particles) used in our studies has the correct (i.e., functional) overall envelope conformation.

Although antibody-mediated gp120-virion dissociation has been proposed as a possible mechanism of neutralization of TCLA isolates (Poignard *et al.,* 1995) our data suggest that this mechanism will not be a predominant one for primary-like HIV-1 isolates. For example, the binding of MAb G3.4 to HIV-1_{SF162} virions resulted in a substantial amount of gp120-virion dissociation (Fig. 1), yet this MAb does not neutralize HIV-1_{SF162} infection (Table 2). Conversely, the anti-V3 loop MAb 391-95D and the anti-CD4 binding site MAb IgG1b12 neutralize infection of HIV-1_{SF162} and HIV-1_{SF128A} effectively (Table 2) without mediating gp120-virion dissociation (Fig. 2 and data not shown). Furthermore, the binding of the anti-V2 loop MAb G3.4, but not MAb G3.136, to the surface of HIV-1_{SF162} virions resulted in gp120-virion dissociation, while the binding of MAb G3.4 to HIV-1_{SF128A} did not (Fig. 1). This suggests that the binding of a given antibody to the same epitope on different isolates may induce conformational changes whose nature and extent will depend on other structural features imposed by the viral genomic background.

What are the mechanisms for neutralization of primary isolates? Data obtained with the anti-V3 loop MAbs tested (Table 2) indicate that the relative degree of neutralization correlates best with their relative extent of binding to their respective epitopes following (or during), but not prior to, virus-sCD4 association (391-95D $\gg$ 257D $\gg$ 268D (Table 1)). This observation suggests that certain functional epitopes (such as that recognized by MAb 391-95D) within the third hypervariable region of gp120 become exposed and accessible to antibody binding, and are targets for neutralization, during the CD4-mediated conformational changes occurring in the envelope gp120, i.e., during HIV-1-cell fusion. With regard to the neutralization by MAb IgG1b12, it is unlikely that its high neutralization potential is simply due to its high affinity for the oligomeric envelope, since its virion-binding relative affinity was only threefold higher (0.1 μg/ml versus 0.3 μg/ml) than that of MAb 654-30D in the HIV-1_{SF162} case, while being identical in the case of HIV-1_{SF128A} (Table 1). We are currently investigating other possible mechanisms by which MAb IgG1b12 might be mediating neutralization of primary-like HIV-1 isolates. It is possible, for example, that virion-binding of this MAb either inhibits virion–cell binding, by blocking the binding of virions to cellular CD4, as stated above, or induces aberrant gp120 structural changes, thus rendering the envelope fusion incompetent. The elucidation of the mechanism by which this MAb mediates neutralization of primary HIV-1 isolates is crucial for the development of effective envelope-based vaccine models.

We consistently observe that MAbs 391-95D and IgG1b12 neutralize HIV-1 infection of human primary macrophage cells more efficiently than infection of PBMC. This could be due to differences in experimental settings for neutralization of these two cell types. However, these conditions, the longer exposure of virus and PBMC to MAbs than virus and macrophages, should favor a more efficient neutralization of PBMC than macro-

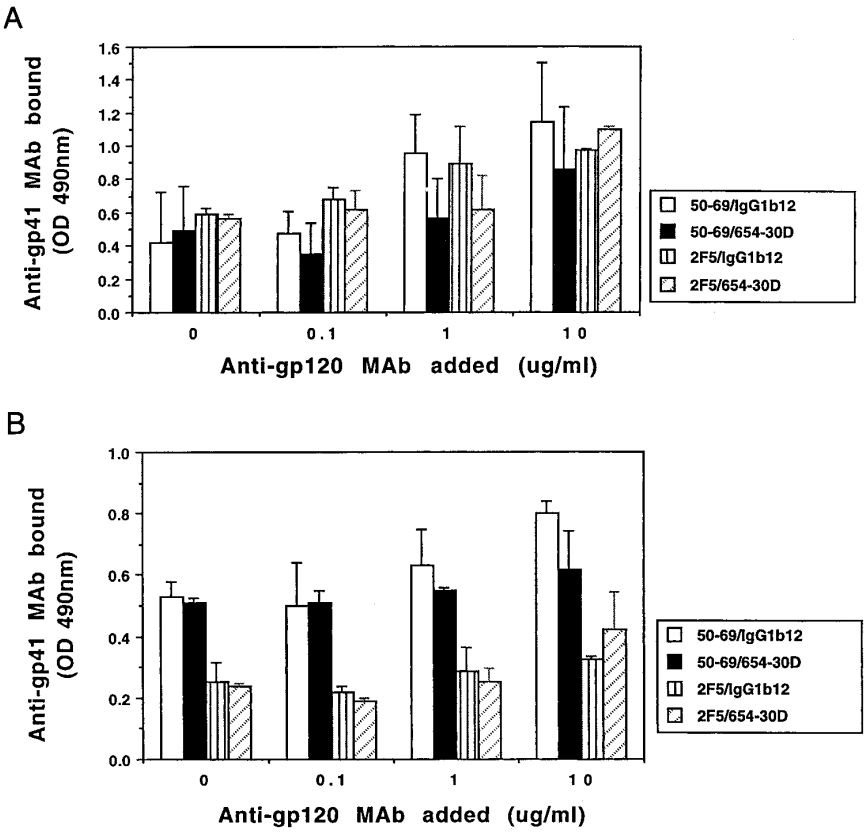

FIG. 4. Exposure of gp41 epitopes occurring upon binding of anti-CD4-binding site MAbs to (A) HIV-1_{SF162} and (B) HIV-1_{SF128A} virions. Sucrose-purified virions were incubated with a constant concentration (1 μg/ml) of human anti-gp41 MAbs (50–69 or 2F5) and increasing concentrations of anti-CD4-binding site MAbs (IgG1b12 or 654-30D). The amounts of anti-gp41 MAbs bound (OD 490 nm) to the virion surface were determined for each concentration of anti-CD4 binding site MAb added, as described under Materials and Methods. The data represent the mean and standard deviation of two independent experiments. The $OD_{490\ nm}$ values shown are corrected for nonspecific MAb binding to the wells (background).

phages. Thus, we believe that the results obtained reflect a true differentiation in the susceptibility of PBMC and macrophages to neutralization. However, it is possible that the neutralization potential of these MAbs is influenced by differences in numbers of target sites present on the two cell types. Macrophages express fewer CD4 molecules than PBMC (Collman *et al.,* 1990) and thus, MAb IgG1b12 might more effectively block the interaction of virions with CD4 molecules present on the former rather than the latter cells. In addition, the consequence of a smaller number of CD4 molecules on the macrophages could be that fewer epitopes recognized by MAb 391-95D will become exposed on virions following virion binding to macrophages, and thus, MAb 391-95D would be more effective in neutralizing infection of macrophages than PBMC. These various possibilities require additional investigation.

Previous data have indicated that binding of the same antibody to two related laboratory-adapted HIV-1 isolates may result in neutralization of infection in one case, but not the other (McKeating *et al.,* 1993). Our data, however, suggest that, in addition to considerations of extent and affinity of binding and gp120-virion dissociation, binding of MAbs to related epitopes may induce opposite effects on viral infectivity. The examples on hand are the anti-CD4-binding site MAbs 654-30D and IgG1b12. Both anti-CD4 binding site MAbs 654-30D and IgG1b12 bind to the virion surface of the two macrophage-tropic isolates tested with high relative affinities (based on half maximal binding values) (Fig. 2 and Table 1). However, MAb IgG1b12 mediates efficient neutralization of infection while MAb 654-30D mediates enhancement of infection when the target cells are primary macrophages (Table 2 and Fig. 3). It is possible that MAb 654-30D enhances HIV-1 infectivity either via the Fc receptors present on the surface of macrophage cells (Homsy *et al.,* 1989; Takeda *et al.,* 1988) or in an Fc-independent fashion (Schutten *et al.,* 1995; Sullivan *et al.,* 1995). Related to this latter, we examined whether specific exposure of gp41 epitopes occurs upon virion-MAb 654-30D binding. Our results, however, failed to reveal any correlation between the extent of exposure of gp41-conformational epitopes recognized by MAb 50-69 and 2F5 with the enhancement of infection by MAbs 654-30D or the neutralization of infection by MAb IgG1b12 (Fig. 4). Nevertheless, since we examined only two gp41 epitopes, the

possibility still remains that MAbs 654-30D and IgG1b12 mediate differential exposure of other gp41 epitopes, e.g., within the fusion peptide, which are implicated in HIV-1-cell entry.

In summary, our studies reveal that the effect that an antibody–virus association will have on HIV-1 infection will depend on the epitope to which the antibody binds on the oligomeric gp120, the genetic background of the virus, and the target cell type. Our data suggest that not every exposed epitope of the immunodominant regions present on gp120 oligomers will be a target for neutralization, that the neutralization potential of MAbs toward primary isolates will not always correlate with their extent or affinity of binding to virion-associated gp120 molecules, and that MAb-mediated gp120-virion dissociation appears not to be a predominant mechanism of neutralization of primary-like HIV-1 isolates. Certain neutralization-sensitive epitopes may be hidden within the oligomeric structure of the HIV-1 envelope prior to the binding of virions to the cell surface, but become exposed during virus–cell fusion. Given this, it is probable that the effectiveness of antibodies targeted to these epitopes in neutralizing infection *in vivo* will depend on the antibody concentration at the site of virus–cell fusion, their epitope binding affinity, and the virus–cell fusion rate constant. This latter factor will probably not only vary among primary HIV-1 isolates, but may be dependent on the cell type and the activation state of the cell. Additional studies using different HIV-1 strains/MAb combinations will allow us to determine whether the above conclusions have a general applicability or whether they are specific for the primary-like isolates used in our studies.

ACKNOWLEDGMENTS

This work was supported by NIH Grants AI29857, AI32424, and AI36085, by the Aaron Diamond AIDS Foundation, PAF Grant 50617-20-PG (L.S.), by an AmFAR Grant made in memory of Bernard C. Hirsh (L.S.), and by general research funds and support from the Research Center for AIDS and HIV Infection from The Department of Veterans Affairs. We thank A. J. Mayer for his critical review of the manuscript.

REFERENCES

Bernier, R., and Tremblay, M. (1995). Homologous interference resulting from the presence of defective particles of human immunodeficiency virus type 1. *J. Virol.* 69, 291–300.

Bou-Habib, D. C., Roderiquez, G., Oravesz, T., Berman, P. W., Lusso, P., and Norcross, M. A. (1994). Cryptic nature of envelope V3 region epitopes protects primary monocytotropic human immunodeficiency virus type 1 from antibody neutralization. *J. Virol.* 68, 6006–6013.

Burton, D. R., Pyati, J., Koduri, R., Sharp, S. J., Thorton, G. B., Parren, P. H. I., Sawyer, L. S. W., Hendry, R. M., Dunlop, N., Nara, P. L., Lamacchia, M., Garratty, E., Stiehm, E. R., Bryson, Y. J., Cao, Y., Moore, J. P., Ho, D. D., and Barbas III, C. F. (1994). Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. *Science* 266, 1024–1027.

Cheng-Mayer, C., Homsy, J., Evans, L. A., and Levy, J. A. (1988). Identification of human immunodeficiency virus subtypes with distinct patterns of sensitivity to serum neutralization. *Proc. Natl. Acad. Sci. USA* 85, 2815–2819.

Cheng-Mayer, C., Quiroga, M., Tung, J. W., Dina, D., and Levy, J. A. (1990). Viral determinants of human immunodeficiency virus type 1 T-cell or macrophage tropism, cytopathogenicity, and CD4 antigen modulation. *J. Virol.* 64, 4390–4398.

Cheng-Mayer, C., Weiss, C., Seto, D., and Levy, J. A. (1989). Isolates of human immunodeficiency virus type 1 from the brain may constitute a special group of the AIDS virus. *Proc. Natl. Acad. Sci. USA* 86, 8575–8579.

Collman, R., Godfrey, B., Cutilli, J., Rhodes, A., Hassan, N. F., Sweet, R., Douglas, D. D., Friedman, H., Nathanson, N., and Gonzalez-Scarano, F. (1990). Macrophage-tropic strains of human immunodeficiency virus type 1 utilize the CD4 receptor. *J. Virol.* 64, 4468–4476.

Fung, M. S. C., Sun, C. R. Y., Gordon, W. L., Liou, R.-S., Chang, T. W., Sun, W. N. C., Daar, E. S., and Ho, D. D. (1992). Identification and characterization of a neutralization site within the second variable region of human immunodeficiency virus type 1 gp120. *J. Virol.* 66, 848–856.

Gorny, M. K., Xu, J.-Y., Karwowska, S., Buchbinder, A., and Zolla-Pazner, S. (1993). Repertoir of neutralizing human monoclonal antibodies specific for the V3 domain of HIV-1 gp120. *J. Virol.* 150, 635–643.

Harrowe, G., and Cheng-Mayer, C. (1995). Amino acid substitutions in the V3 loop are responsible for adaptation to growth in transformed T-cell lines of a primary human immunodeficiency virus type 1. *Virology* 210, 490–494.

Homsy, J., Meyer, M., Tateno, M., Clarkson, S., and Levy, J. A. (1989). The Fc and not CD4 receptor mediates antibody enhancement of HIV infection in human cells. *Science* 244, 1357–1360.

Koito, A., Stamatatos, L., and Cheng-Mayer, C. (1995). Small amino acid sequence changes within the V2 domain can affect the function of a T-cell line-tropic human immunodeficiency virus type 1 envelope gp120. *Virology* 206, 878–884.

Laal, S., Burda, S., Gorny, M. K., Karwowska, S., Buchbinder, A., and Zolla-Pazner, S. (1994). Synergistic neutralization of human immunodeficiency virus type 1 by combinations of human monoclonal antibodies. *J. Virol.* 68, 4001–4008.

Liu, Z.-Q., Wood, C., Levy, J. A., and Cheng-Mayer, C. (1990). The viral envelope gene is involved in macrophage tropism of a human immunodeficiency virus type 1 strain isolated from brain tissue. *J. Virol.* 64, 6148–6153.

McKeating, J. A., Bennett, J., Zolla-Pazner, S., Schutten, M., Ashelford, S., Brown, D. D., and Balfe, P. (1993). Resistance of a human serum-selected human immunodeficiency virus type 1 escape mutant to neutralization by CD4 binding site monoclonal antibodies is conferred by a single amino acid change in gp120. *J. Virol.* 67, 5216–5226.

Moore, J. P., McKeating, J. A., Huang, Y., Ashkenazi, A., and Ho, D. D. (1992). Virions of primary human immunodeficiency virus type 1 isolates resistant to soluble CD4 (sCD4) neutralization differ in sCD4 binding and glycoprotein gp120 retention from sCD4-sensitive isolates. *J. Virol.* 66, 235–243.

Moore, J. P., Cao, Y., Qing, L., Sattentau, Q. J., Pyati, J., Koduri, R., Robinson, J., Barbas III, C. F., Burton, D. R., and Ho, D. D. (1995). Primary isolates of human immunodeficiency virus type 1 are relatively resistant to neutralization by monoclonal antibodies to gp120, and their neutralization is not predicted by studies with monomeric gp120. *J. Virol.* 69, 101–109.

Moore, J. P., Sattentau, Q. J., Yoshiyama, H., Thali, M., Charlles, M., Sullivan, N., Poon, S.-W., Fung, M. S., Traincard, F., Pinkus, M., Robey, G., Robinson, J. E., Ho, D. D., and Sodroski, J. (1993). Probing the structure of the V2 domain of human immunodeficiency virus type 1 surface glycoprotein gp120 with a panel of eight monoclonal antibodies: Human immune response to the V1 and V2 domains. *J. Virol.* 67, 6136–6151.

Moore, J. P., and Ho, D. D. (1995). HIV-1 neutralization: The conse-

quences of viral adaptation to growth on transformed T cells. *AIDS* 9, 117–136.

Muster, T., Steindl, F., Purtscher, M., Trkola, A., Klima, A., Himmler, G., Rüker, F., and Katinger, H. (1993). A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1. *J. Virol.* 67, 6642–6647.

Poignard, P., Fouts, T., Naniche, D., Moore, J. P., and Sattentau, Q. J. (1995). Neutralizing antibodies to human immunodeficiency virus type-1 gp120 induce envelope glycoprotein subunit dissociation. *J. Exp. Med.* 183, 473–484.

Sattentau, Q. J., and Moore, J. P. (1995). Human immunodeficiency virus type 1 neutralization is determined by epitope exposure on the glycoprotein gp120 oligomer. *J. Exp. Med.* 182, 185–196.

Sawyer, L. S. W., Wrin, T., Crawford-Miksza, L., Potts, B., Wu, Y., Weber, P. A., Alfonso, R. D., and Hanson, C. V. (1994). Neutralization sensitivity of human immunodeficiency virus type 1 is determined in part by the cell in which the virus is propagated. *J. Virol.* 68, 1342–1349.

Schutten, M., Andeweg, A. C., Bosch, M. L., and Osterhaus, A. D. M. E. (1995). Enhancement of infectivity of a non-syncytium inducing HIV-1 by sCD4 and by human antibodies that neutralize syncytium inducing HIV-1. *Scand. J. Immunol.* 41, 18–22.

Stamatatos, L., and Cheng-Mayer, C. (1993). Evidence that the structural conformation of the envelope gp120 affects human immunodeficiency virus type 1 infectivity, host range, and syncytium-forming ability. *J. Virol.* 67, 5635–5639.

Stamatatos, L., and Cheng-Mayer, C. (1995). Structural modulations of the envelope gp120 glycoprotein of human immunodeficiency virus type 1 upon oligomerization and differential V3 loop epitope exposure of isolates displaying distinct tropism upon virion-soluble receptor binding. *J. Virol.* 69, 6191–6198.

Stamatatos, L., Werner, A., and Cheng-Mayer, C. (1994). Differential regulation of cellular tropism and sensitivity to soluble CD4 neutralization by the envelope gp120 of human immunodeficiency virus type 1. *J. Virol.* 68, 4973–4979.

Sullivan, N., Sun, Y., Li, J., Hofmann, W., and Sodroski, J. (1995). Replicative function and neutralization sensitivity of envelope glycoproteins from primary and T-cell line-passaged human immunodeficiency virus type 1 isolates. *J. Virol.* 69, 4413–4422.

Takeda, A., Tuazon, C. U., and Ennis, F. A. (1988). Antibody-enhanced infection by HIV-1 via Fc receptor-mediated entry. *Science* 242, 580–583.

VanCott, T. C., Bethke, F. R., Polonis, V. R., Gorny, M. K., Zolla-Pazner, S., Redfield, R. R., and Birx, D. (1994). Dissociation rate of antibody-gp120 binding interactions is predictive of V3-mediated neutralization of HIV-1. *J. Immunol.* 153, 449–459.